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Attorney Docket No. 23908-501


**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

APPLICANT: Goldshtein

SERIAL NUMBER: 09/966,847 EXAMINER: Patrick T. Lewis

FILING DATE: September 28, 2001 ART UNIT: 1623

FOR: HYDROPHILIC COMPLEXES OF LIPOPHILIC MATERIALS AND AN APPARATUS AND METHOD FOR THEIR PRODUCTION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

# DECLARATION UNDER 37 C.F.R. § 1.132

I, Rina Goldshtein, of 157, Shani, Har Hebron, 90411, Israel hereby declare and state as follows:

1. I am the sole inventor for the United States patent application entitled "Hydrophilic Complexes of Lipophilic Materials and an Apparatus and Method for their Production", serial number 09/966,847, which was filed September 28, 2001.

2. I am employed by Solubest Ltd., the assignee of this application. My title is Chief Scientist. I received a Ph.D. in Chemistry from Leningrad State University in 1983, where I studied organic/polymer chemistry and mechanisms of reactions. I have been with Solubest from 2001 to the present.

3. I have reviewed the June 30, 2003 Office Action. In particular, I understand that the Examiner has rejected pending claims 1-18 and 33-49 under 35 U.S.C. §112, first paragraph, contending that the pending claims do not comply with the written description requirement.

4. I make this declaration to rebut the Examiner's assertion, with which I do not agree. It is my opinion that the pending claims meet the written description requirement of 35 U.S.C. §112, first paragraph. The Examiner states that there is insufficient written description with respect to vitamins, antibiotics, hormones and polysaccharides. To the contrary, one of ordinary skill in the art can readily determine what is encompassed by these terms and determine, from reading the specification, that the claimed invention encompasses members within these groups.

5. To illustrate this I enclose the following experimental results. I have performed, or have had performed under my supervision, studies evaluating the formation of hydrophilic inclusion complexes where these complexes include nano-sized lipophilic particles surrounded by an amphiphilic polymer where the resulting inclusion complex is rendered hydrophilic in water. These studies have been conducted with various lipophilic particles (representative results have been described for several particles, *i.e.* antibiotics, anti-fungal agent and anti-cancer agent) and various amphiphilic polymers guided by the matching parameter teachings described in the instant application.

6. In these studies, we used the major parameters of the lipophilic compound and the polymer, as described in the instant application (*i.e.*, HLB (hydrophilic-lipophilic balance), length of the polymer chain, flexibility of the polymer chain, state of polarity of the hydrophilic groups, quantity of the polymer fragments capable of participating in the complex creating groups in the basic chain, functional groups capable for complex creating with specific active compound, degree of its modification, if it is modified; and molecular mass) for formation of a hydrophilic inclusion complexes that is water soluble.

7. In a first study, results show that when the macrolide antibiotic, Clarithromycin, which is a poorly soluble hydrophobic compound, is surrounded by a polymer the resulting inclusion complex is hydrophilic. Using the matching technique described in the instant application, Clarithromycin is rendered hydrophilic when surrounded by various polymers which meet the matching parameters; such as, Alginat, PVA and Chitosan (*See*, Appendix A). The results show that 2% Alginat combined with 10mg/ml of Clarithromycin at pH 5.5 resulted in nanoparticles with a ALV size distribution analyses of 530 nm (93%); 2% PVA combined with Clarithromycin at pH 6 resulted in nanoparticles with a ALV of 1600 nm (100%); 1% Chitosan (Fluka) combined with Clarithromycin at pH 4-6 resulted in a ALVof 165 nm (100%); 1% Chitosan (Fluka) combined with Clarithromycin at pH 4 resulted in an ALV of 321 nm (100%); 1% Chitosan (Fluka) combined with Clarithromycin at pH 6 resulted in an ALV of 660 nm (100%); and 1% Chitosan (Sigma) combined with Clarithromycin at pH 5 resulted in an ALV of 838 nm (100%). These results show that using the teachings of the specification (lipophilic/amphiphilic polymer matching technique) a poorly soluble hydrophobic antibiotic

(Clarithromycin) can be surrounded by various amphiphilic polymers (i.e. Alginat, PVA and Chitosan) to render the resulting inclusion complex hydrophilic in water.

8. In a second study, results show that when the macrolide antibiotic, Azithromycin, which is also a poorly soluble hydrophobic compound, is surrounded by a polymer the resulting inclusion complex is hydrophilic. Using the matching technique described in the instant application, Azithromycin is rendered hydrophilic when surrounded by various polymers which meet the matching parameters; such as, Alginat, PVA, Manucol Ester B, and Chitosan (*See*, Appendix B). The results show that 2% PVA combined with 10mg/ml of Azithromycin resulted in a nanoparticle size distribution analyses of ALV of 5 nm (98%); 4% Manucol Ester B combined with Azithromycin resulted in an ALV of 330 nm(100%); 1% PVA combined with Azithromycin resulted in an ALV of 5 nm (95%) and in another formulation process, 350 nm (100%); 2% Alginat combined with Azithromycin resulted in an ALV of 1600 nm (100%) and in another formulation process 1060 nm (100%), 1% Chitosan (Sigma) combined with Azithromycin resulted in a ALVof 510 nm (100%), and in other formulation processes :752 nm (100%) and 362 nm (100%). These results are consistent with the results of the first study and show that using the teachings of the specification (lipophilic/amphiphilic polymer matching technique) a poorly soluble hydrophobic antibiotic (Azithromycin) can be surrounded by various amphiphilic polymers (*i.e.* Alginat, PVA, Manucol Ester B, and Chitosan) to render the resulting inclusion complex hydrophilic in water.

9. In a third study, results show that when the anti-fungal agent, Itraconazole, which is an insoluble compound, is surrounded by a polymer the resulting inclusion complex is hydrophilic. Using the matching technique described in the instant application, Itraconazole is rendered hydrophilic when surrounded by various polymers which meet the matching parameters; such as, Thermo-destructed Starch combined with $H_2O_2$ and PEG, Alginat, and Chitosan (*See*, Appendix C). The results show that 5% Thermo-destructed Starch + 1.25% $H_2O_2$ + 1.25% PEG combined with 5 mg/ml of Itraconazole resulted in an ALV of 382 nm (100%); 5% Thermo-destructed Starch + 0.625% $H_2O_2$ + 1.25% PEG combined with 5 mg/ml of Itraconazole resulted in an ALV of 640 nm (100%); 5% Thermo-destructed Starch + 1% $H_2O_2$ + 2% PEG combined with 5 mg/ml of Itraconazole resulted in an ALV of 414 nm (100%); 5% Thermo-destructed Starch + 1% $H_2O_2$ + 1% PEG combined with 5 mg/ml of Itraconazole resulted in an ALV of 793 nm

(100%); 2% Alginat combined with 20 mg/ml of Itraconazole resulted in an ALV of 180 nm (100%) and when combined with 10 mg/ml of Itraconazole resulted in an ALV of 180 nm (100%); 1% Chitosan (Fluka) combined with ~8 mg/ml Itraconazole resulted in an ALV of 120 nm (100%). These results show that using the teachings of the specification (lipophilic/amphiphilic polymer matching technique) an insoluble anti-fungal agent (Itraconazole) can be surrounded by various amphiphilic polymers (*i.e.* Thermo-destructed Starch combined with $H_2O_2$ and PEG, Alginat, and Chitosan) to render the resulting inclusion complex hydrophilic in water.

10. In a fourth study, results show that when the anti-cancer agent, Taxol, which is an insoluble compound, is surrounded by a polymer the resulting inclusion complex is hydrophilic. Using the matching technique taught by the instant application, Taxol at various concentrations (0.872 mg/ml, 0.646 mg/ml, 3.781 mg/ml and 0.925 mg/ml) is rendered hydrophilic when surrounded by the polymer gelatin (with added vitamin B12 excipient) which meets the matching parameters described in the instant application (*See*, Appendix D).

11. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Rina Goldshtein

Signed at Rehovot, Israel
this 29 day of September, 2003

## Appendix A

### Clarithromycin Formulations

Table 1 below shows the results of the formation of a Clarithromycin Hydrophilic Inclusion Complexes. The numerous formulations were carried out following the teachings of the instant application and are based on the applied experimental conditions (% polymer, pH, drug concentration). The table also shows the physico-chemical analysis of the various formulations, noted are ALV-size and size distribution, HPLC (concentration and thus solubility) and in some formulations powder X-ray analyses for the determination of crystalline phase.

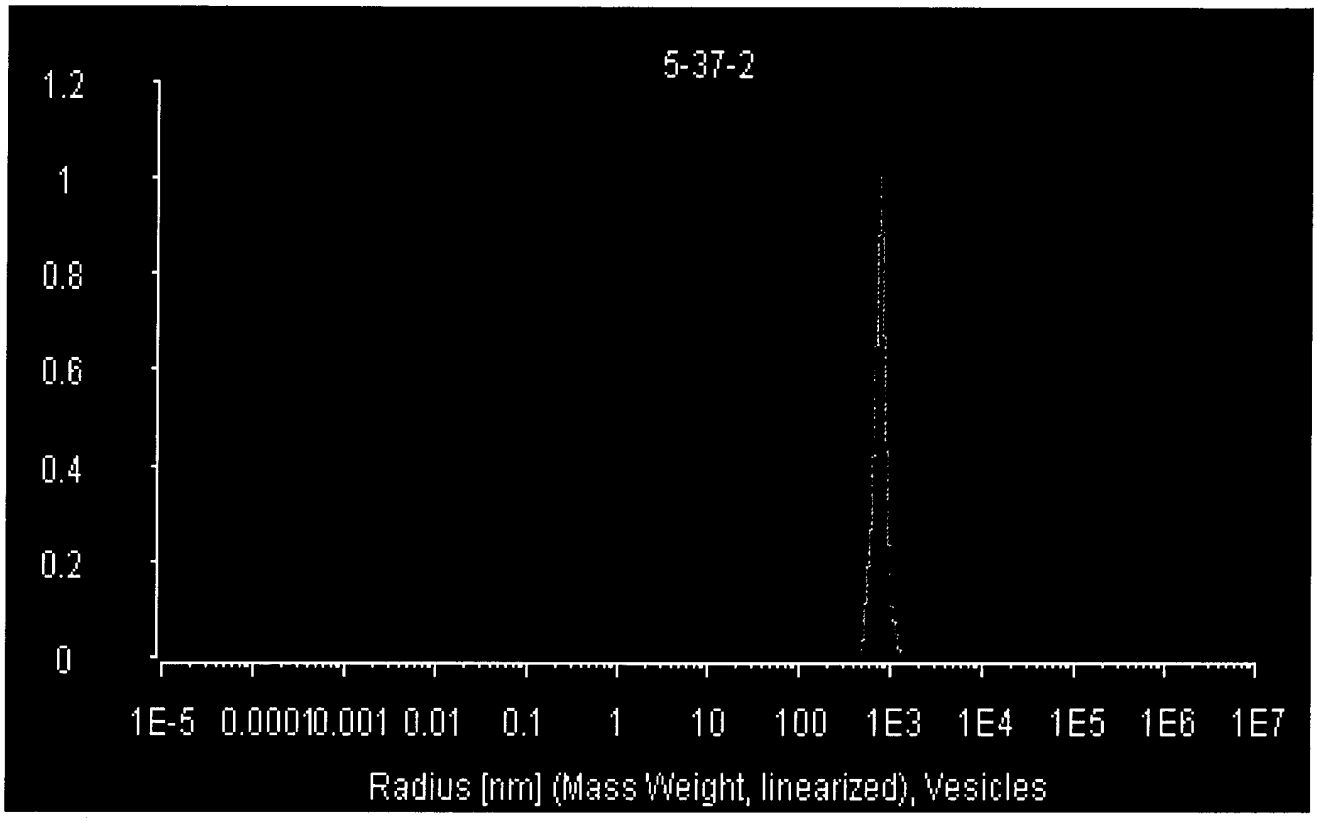
**Table 1.**

| | | | | HPLC | | | | |
|---|---|---|---|---|---|---|---|---|
| **Exp.** | **Polymer (name/%)** | **Drug (mg/ml)** | **pH** | **Quantity (ml)** | **Total IT (mg/ml)** | **% from Initial** | **ALV nm** | **X-Ray** |
| IC-98 (75) **1.10.02** | Hydrolized potato starch 4% dil to 2% | 10 | 5 | 5 | 9.39 | 93.9 | | |
| IC-133 **9.12.02** | 2% Alginat Kelton LV | 10 | 5.5 | 20 | 4.43 | 44.3 | **530 (93%)** | |
| IC-135 **15.12.02** | 1% Chitosan **Fluka 50494** | 10 | 4 - 6 | 5 | 8.45 | 84.5 | **165 (100%)** | Amorphous |
| 1Cl-IZ-10-34 **2.01.03** | 2% PVA | 10 | 6 | 35 | 5.82 | 76.9 | **1600 (100%)** | Crystalline |
| 1Cl-IZ-135/1-10-112 **11.05.03** | 1% Chitosan **Fluka 50494** | 10 | 4 | | 9.13 | 91.3 | **321 (100%)** | |
| 1Cl-IZ-135/1-10-112 **11.05.03** | 1% Chitosan **Fluka 50494** | 10 | 6 | | 9.18 | 99.6 | **660 (100%)** | |
| 1Cl-IZ-135/2-10-134 **15.06.03** | 1% Chitosan **Sigma C3646** | 10 | 5 | No | 6.3 | 55.4 | **838 (100%)** | |

HPLC = High Performance Liquid Chromatography

Figure 1 below shows the results of ALV Nano-Size Particle Analysis for a Clarithromycin Hydrophilic Inclusion Complex (Formulation 10-134). ALV is a dynamic light scattering technique used to estimate the mean particle size. Experiments were conducted with a laser powered Noninvasive Back Scattering=High Performance Particle Sizer – ALV-NIBS/HPPS (ALV, Langen, Germany – ALV-Laser Vertriebsgesellschaft m.b.H. Rober-Bosch-Strabe 46, D-63225 Langen/Germany).

**Figure 1.**




ALV-Correlator Distribution Function Fit

Fitted at 8/27/03 11:56:49 AM
Sample Name :
Degrees of freedom found : 3
Unregularized Fit Error : 6.4191362E-03
Regularized Fit Error : 6.4804671E-03

Baseline fitted as : 0.0000000E+00
Radius, Mass Weight, linearized

Peak 1, from 6.189E+002 [nm] to 1.000E+003 [nm]
Weight of Peak [%] : 100.00000
Mean Peak Position : 7.634E+002 [nm]
Relative Peak Width : ± 1.277E-001

Moments of entire Solution
Total Weight of Peaks [%] : 100.00000
Mean Position of Peaks : 7.634E+002 [nm]
Relative Peak Width : ± 1.277E-001

## Appendix B

### Azithromycin Formulations

Table 2 below shows the results of the formation of a Azithromycin Hydrophilic Inclusion Complexes. The numerous formulations were carried out following the teachings of the instant application and are based on the applied experimental conditions (% polymer, pH, drug concentration). The table also shows the physico-chemical analysis of the various formulations, noted are ALV-size and size distribution, HPLC (concentration and thus solubility) and in some formulations powder X-ray analyses for the determination of crystalline phase.

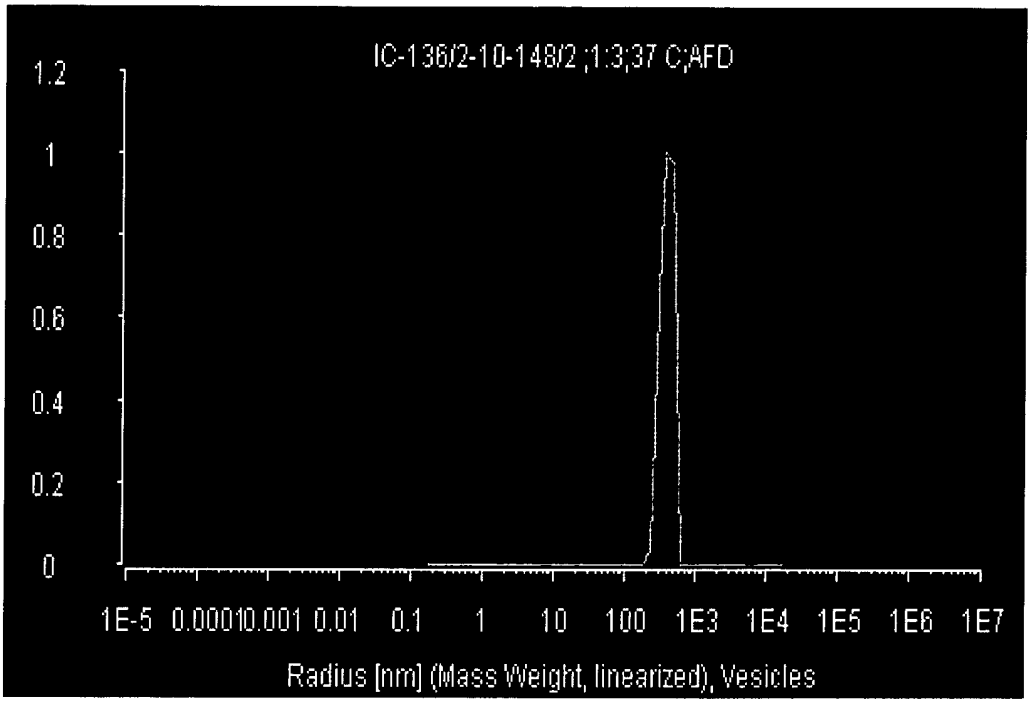
**Table 2.**

| | | | HPLC | | |
|---|---|---|---|---|---|
| **Exp.** | **Polymer (name/%)** | **Drug (mg/ml)** | **Total IT (mg/ml)** | **% from Initial** | **ALV nm** |
| 2AZ-IZ-10-32 **2.01.03** | 2% PVA | 10 | 9.9 | 99 | **5 (98%)** |
| 2AZ-IZ-10-36 **06.01.03** | 4% Manucol Ester B | 10 | 9.04 | 90.4 | **330 (100%)** |
| 2AZ-IZ-10-42 **09.01.03** | 1% PVA | 10 | 9.4 (af.filtr.) | 94 | **5 (95%)** |
| AZ-IC-131/1-IZ-10-145 **30.07.03** | 2% Alginat Kelton LV | 20 | 15.58 | 82.6 | **1600 (100%)** |
| AZ-IC-10-42/1-IZ-10-146 **31.07.03** | 1% PVA | 10 | 9.2 | 99.32 | **350 (100%)** |
| AZ-IC-134/1-IZ-10-147 **4.08.03** | 2% Alginat Kelton LV | 10 | 9.25 | 98.06 | **1060 (100%)** |
| AZ-IC 136/2-IZ-10-148 **5.08.03** | 1% Chitosan **Sigma C3646** | 10 | 9.16 | 97.16 | **510 (100%)** |
| AZ-IC 136/3-IZ-28-1 **11.08.03** | 1% Chitosan **Sigma C3646** | 10 | 9.17 | 95.36 | **752 (100%)** |
| AZ-IC 136/2-10-148/2 **5.08.03** | 1% Chitosan **Sigma C3646** | 10 | 9.16 | 97 | **362 (100%)** |

HPLC = High Performance Liquid Chromatography

Figure 2 below shows the results of ALV Nano-Size Particle Analysis for a Azithromycin Hydrophilic Inclusion Complex (Formulation 10-148/2). ALV is a dynamic light scattering technique used to estimate the mean particle size. Experiments were conducted with a laser powered Noninvasive Back Scattering=High Performance Particle Sizer – ALV-NIBS/HPPS (ALV, Langen, Germany – ALV-Laser Vertriebsgesellschaft m.b.H. Rober-Bosch-Strabe 46, D-63225 Langen/Germany).

**Figure 2.**




ALV-Correlator Distribution Function Fit

Fitted at 8/27/03 11:14:24 AM
Sample Name :
Degrees of freedom found : 4
Unregularized Fit Error : 8.5468653E-03
Regularized Fit Error : 8.6631877E-03

Baseline fitted as : 4.9103406E-03
Radius, Mass Weight, linearized

Peak 1, from 2.371E+002 [nm] to 4.870E+002 [nm]
Weight of Peak [%] : 100.00000
**Mean Peak Position : 3.925E+002 [nm]**
Relative Peak Width : ± 1.932E-001

Moments of entire Solution
Total Weight of Peaks [%] : 100.00000
Mean Position of Peaks : 3.925E+002 [nm]
Relative Peak Width : ± 1.932E-001

## Appendix C

### Itraconazole Formulations

Table 3 below shows the results of the formation of a Itraconazole Hydrophilic Inclusion Complexes. The numerous formulations were carried out following the teachings of the instant application and are based on the applied experimental conditions (% polymer, pH, drug concentration). The table also shows the physico-chemical analysis of the various formulations, noted are ALV-size and size distribution, HPLC (concentration and thus solubility) and in some formulations powder X-ray analyses for the determination of crystalline phase.

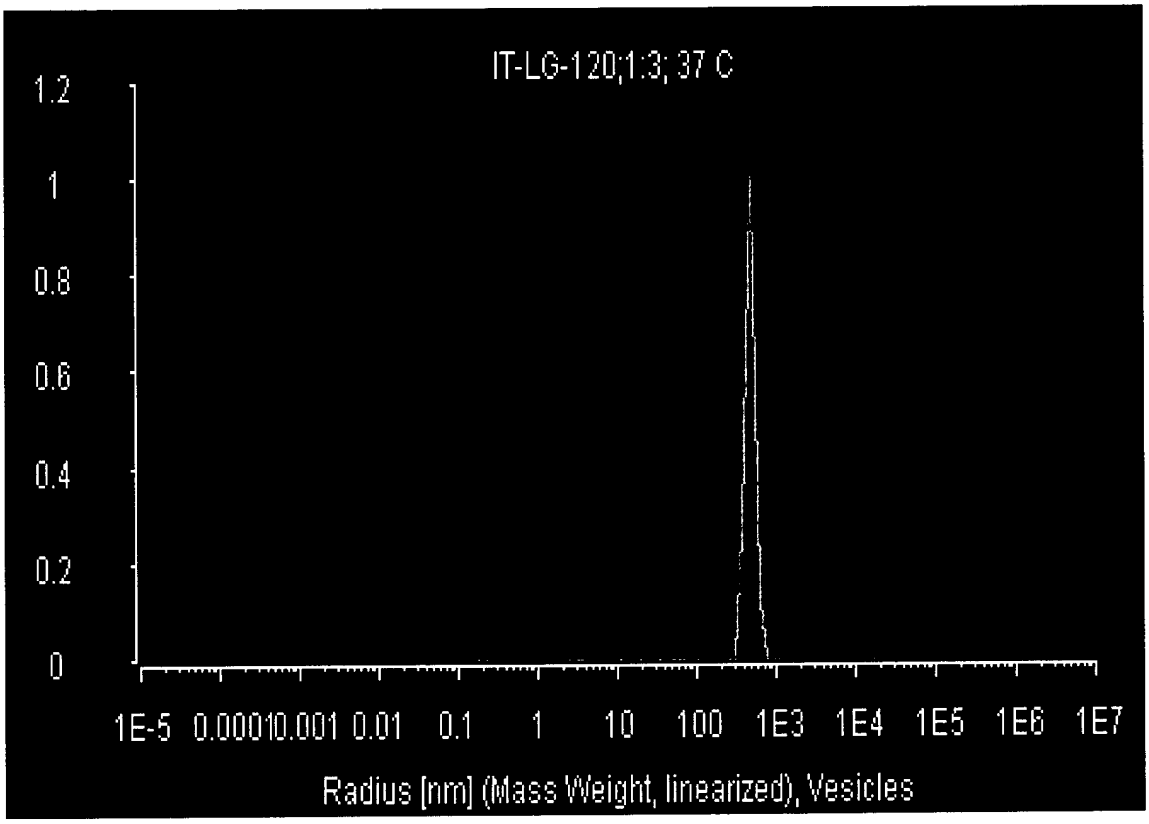
**Table 3.**

| | | | HPLC | | |
|---|---|---|---|---|---|
| **Exp.** | **Polymer (name/%)** | **Drug (mg/ml)** | **Total IT (mg/ml)** | **% from Initial** | **ALV nm** |
| 07IT-IZ-10-91 **24.03.03** | 5% Hydrolized potato starch + 1% $H_2O_2$ | 2 | 1.676 (d.) | 83.8 | |
| 07IT-IZ-10-105 **28.04.03** | 4% Hydrolized potato starch + 1% PEG | 5 | 4.79 | 86.28 | |
| 07IT-IZ-10-140 **2.07.03** | 1% Chitosan **Sigma C3646** | 5 | | | |
| 7IT-LG-23-104 **21.07.03** | 5% Thermo-destructed starch + 1.25% $H_2O_2$ + 1.25% PEG | 5 | 5.09 | 101.8 | **382 (100%)** |
| 7IT-LG-23-112 **23.07.03** | 5% Thermo-destructed starch + 0.625% $H_2O_2$ + 1.25% PEG | 5 | 4.97 | 99.5 | **640 (100%)** |
| 7IT-LG-23-120 **30.07.03** | 5% Thermo-destructed starch + 1% $H_2O_2$ + 2% PEG | 5 | 5.016 | 100 | **414 (100%)** |
| 7IT-LG-23-113 **27.07.03** | 5% Thermo-destructed starch + 1% $H_2O_2$ + 1% PEG | 5 | 4.52 | 90.41 | **793 (100%)** |
| IC-131 **05.12.02** | 2% Alginat Kelton LV | 20 | 20.2 | 101 | **180 (100%)** |
| IC-134 **11.12.02** | 2% Alginat Kelton LV | 10 | 9.5 | 95 | **1250 (100%)** |
| IC-136 **18.12.02** | 1% Chitosan **Fluka 50494** | ~ 8 | 8.2 | 100 | **120 (100%)** |

HPLC = High Performance Liquid Chromatography

Figure 3 below shows the results of ALV Nano-Size Particle Analysis for a Itraconazole Hydrophilic Inclusion Complex (Formulation 23-120). ALV is a dynamic light scattering technique used to estimate the mean particle size. Experiments were conducted with a laser powered Noninvasive Back Scattering=High Performance Particle Sizer – ALV-NIBS/HPPS (ALV, Langen, Germany – ALV-Laser Vertriebsgesellschaft m.b.H. Rober-Bosch-Strabe 46, D-63225 Langen/Germany).

**Figure 3.**




ALV-Correlator Distribution Function Fit

Fitted at 8/21/03 11:11:41 AM
Sample Name :
Degrees of freedom found : 2
Unregularized Fit Error : 1.3098676E-02
Regularized Fit Error : 1.3171722E-02

Baseline fitted as : 0.0000000E+00
Radius, Mass Weight, linearized

Peak 1, from 3.831E+002 [nm] to 6.189E+002 [nm]
Weight of Peak [%] : 100.00000
Mean Peak Position : 4.745E+002 [nm]
Relative Peak Width : ± 1.245E-001

Moments of entire Solution
Total Weight of Peaks [%] : 100.00000
Mean Position of Peaks : 4.745E+002 [nm]
Relative Peak Width : ± 1.245E-001

ALV-Correlator Distribution Function Fit

Fitted at 8/27/03 11:56:49 AM
Sample Name :
Degrees of freedom found : 3
Unregularized Fit Error : 6.4191362E-03
Regularized Fit Error : 6.4804671E-03

Baseline fitted as : 0.0000000E+00
Radius, Mass Weight, linearized

Peak 1, from 6.189E+002 [nm] to 1.000E+003 [nm]
Weight of Peak [%] : 100.00000
Mean Peak Position : 7.634E+002 [nm]
Relative Peak Width : ± 1.277E-001

Moments of entire Solution
Total Weight of Peaks [%] : 100.00000
Mean Position of Peaks : 7.634E+002 [nm]
Relative Peak Width : ± 1.277E-001

## Appendix D

### Taxol Formulations

Table 4 below shows the results of the formation of a Taxol Hydrophilic Inclusion Complexes. The numerous formulations were carried out following the teachings of the instant application and are based on the applied experimental conditions (amount of excipient $B_{12}$ with polymer and drug concentration). The table also shows the physico-chemical analysis of the various formulations, noted are ALV-size and size distribution. HPLC (concentration and thus solubility) results determined the max taxol concentration.

**Table 4.**

| Batch | $B_{12}$ Conc. in polymer solution (mg/ml) | Max Taxol Conc. (mg/ml) | Particle Size (nm) |
|---|---|---|---|
| 5TX-OS-25-85 | 1 | 0.872 | 179 |
| 5TX-OS-25-76 | 0 | 0.646 | 117 |
| 5TX-OS-25-89 | 1 | 3.781 | 129 |
| 5TX-OS-25-80 | 1 | 0.925 | 186 |

Figure 4 below shows the results of ALV Nano-Size Particle Analysis for a Taxol Hydrophilic Inclusion Complex (Formulation 25-85). ALV is a dynamic light scattering technique used to estimate the mean particle size. Experiments were conducted with a laser powered Noninvasive Back Scattering=High Performance Particle Sizer – ALV-NIBS/HPPS (ALV, Langen, Germany – ALV-Laser Vertriebsgesellschaft m.b.H. Rober-Bosch-Strabe 46, D-63225 Langen/Germany).

**Figure 4.**